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Some fungi associated with Collembola[1]

BY

Alice A. CHRISTEN

Department of Plant Pathology, Washington State University, Pullman, Washington 99163

INTRODUCTION

Collembola play a significant role in the breakdown of organic debris and several workers have reported fungi in the guts of these arthropods (ANDERSON *et al.*, 1972 ; BÖDVARSSON, 1970 ; GILMORE and RAFFENSPERGER, 1970 ; MACMILLAN and HEALEY, 1971). Other more detailed studies have involved gut analyses of several species of Collembola, and various fungal structures have been found intact or in fragments, e.g. Basidiomycete hyphae, *Mucor*-type zygospores, conidia, conidiophores and hyphae of Fungi Imperfecti (ANDERSON *et al.*, 1972 ; FRANKLAND, 1966 ; HEALEY, 1965 ; TALBOT, 1952). Specific identification of fungi in slide-mounted Collembola, however, is generally impossible. A few workers have isolated and identified some of the fungi found in and on Collembola; a continuation of these efforts (KNIGHT and ANGEL, 1967; SINGH, 1970) with four species of Collembola is reported here.

MATERIALS AND METHODS

Onychiurus cocklei (Folsom) and *Hypogastrura matura* (Folsom) were obtained from soil samples collected 9 February 1973 from a wheat field at St. John, Washington. *Onychiurus* sp. *fimetarius*-group was obtained from rabbit dung, Spokane, Washington, and *Hypogastrura macgillivrayi* (Folsom) from around building foundations, Packwood, Washington.

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Materials containing the arthropods were stored in plastic containers at 5° C until processed. Soil samples were placed in Berlese (Tullgren) separator funnels (BEIRNE, 1955) for extraction into 95 % ethyl alcohol, or into sterile water for live collection of specimens used for fungal isolations. The extraction apparatus was modified using 60, 40 or 25 watt bulbs, with a 30 cm funnel. Fifty percent lactic acid was used as a temporary mounting medium, and slides were warmed overnight at 50° C prior to preliminary microscopic examination of specimens of *Onychiurus* sp. *fimetarius*-group.

Aseptic techniques were used in fungal isolation procedures. Surface sterilization was performed to help distinguish between external and internal fungi and was accomplished with a 50:50 95 % ethyl alcohol and Chlorox (5.25 % sodiumhypochlorite) treatment for 5 or 10 sec utilizing a Buchner funnel for *Onychiurus* sp. *fimetarius*-group and *Hypogastrura macgillivrayi*. Specimens of *Hypogastrura matura* were surface treated by swirling in a 20 ppm available chlorine solution for 20 min (H. J. Jensen, Oregon State University, personal communication) followed by a sterile water rinse. Treated and untreated or control Collembola were plated on potato dextrose agar (PDA), PDA plus 100 ppm streptomycin sulfate, PDA plus three drops of 25 % lactic acid per 100 ml of media, Sabouraud agar diluted to one-third strength, and water agar. Growing fungal colonies were isolated from five substrate categories : (I) plated collembolan bodies ; (II) exuvia of live Collembola that molted on the agar plate ; (III) tracks made by live individuals on agar media ; (IV) agar substrate in areas where tracks or excretion material were indistinguishable ; (V) secreted or egested material. Pure cultures were established by hyphal tipping and stocks made for subsequent identifications.

RESULTS AND DISCUSSION

Dematiaceous hyphae and various kinds of spores were found singly or in clumps in the gut contents of all 120 mounted individuals of *Onychiurus* sp. *fimetarius*-group. Some spores were multiseptate and provisional indentifications could be made. Culturing was necessary to determine the fungi more precisely.

Thirty isolations were made from specimens of *Onychiurus* sp. *fimetarius*-group and 118 isolations from 320 specimens of *Hypogastrura macgillivrayi*. Untreated Collembola produced 83 % of the total colonies; the remaining 17 % grew from surface sterilized individuals. Surface sterilized Collembola were killed in the process, whereas some of the control specimens remained alive and provided more categories for fungal isolations. All isolates were identified and assigned to 30 different species. Fungal species, site of isolation, treated or untreated Collembola, and collembolan species are recorded (Table I).

Individuals of *Hypogastrura macgillivrayi* that remained alive on the isolation plates fed on developing colonies of *Cladosporium herbarum*, *Penicillium* sp., *Fusarium roseum* ' *Avenaceum* ', *Trichosporon* sp., *Epicoccum nigrum*, and *Aureobasidium* sp. In preliminary feeding tests, ingested spores of *Phoma* sp. appeared to be rendered nonviable by *Sinella curviseta* (CHRISTEN, 1974).

In control treatments of *H. macgillivrayi* at least 25 % of the individuals underwent ecdysis within 14 hours and thus exhibited no gut contents

TABLE I

Fungi isolated from collembolan bodies, products, and associated substrates

Collembolan and fungal species	Site of isolation				
	Collembolan proper	Exuvia	Tracks	Substrate	Secreted or emitted material
1) *Onychirus* sp. *fimetarius*-group					
Phycomycetes					
Conidiobolus coronatus (Cost.) and Srin. and Thirum.				X	
Conidiobolus sp.	X			X	X
Mortierella humilis Linnemann	X				
Fungi Imperfecti					
****Chrysosporium tropicum* Carmichael	X				
**Penicillium atramentosum* Thom	X				
****Phialophora* sp.	X				
Scopulariopsis asperula (Sacc.) Hughes	X				
**Scopulariopsis brevicaulis* (Sacc.) Bainier	X				
****Wardomyces anomala* Brooks and Hansford	X				
****Yeast*	X				
2) *Hypogastrura macgillivrayi* (Folsom)					
Phycomycetes					
Absidia californica Ellis and Hesseltine		X			
Mortierella ramanniana (Möller) Linnemann				X	
Mucor varians Povah			X		
Fungi Imperfecti					
Aureobasidium sp.	X				
****Bactrodesmium traversianum* (Peyronel) M. B. Ellis	X				
Beauveria brongniartii (Sacc.) Petch	X			X	
**Chrysosporium pannorum* (Link) Hughes	X		X		
Chrysosporium tropicum Carmichael	X				
**Cladosporium herbarum* (Pers.) Link ex S. F. Gray	X	X	X	X	
Epicoccum nigrum Link				X	
Fusarium roseum Lk. emend. Snyd. and Hans '*Avenaceum*'			X	X	
**Paecilomyces farinosus* (Dicks. ex Fr.) A. H. S. Brown and G. Smith	X				
Penicillium canescens Sopp	X				

Collembolan and fungal species	Site of isolation				
	Collembolan proper	Exuvia	Tracks	Substrate	Secreted or emitted material
**Pestalotia truncata* Lév.	X		X		
Phoma sp.				X	
**Thysanophora penicilloides* (Roumeguère) Kendrick	X			X	
Trichosporon sp.					X
Verticillium lecanii (Zimm.) Viégas	X				
3) *Hypogastrura matura* (Folsom)					
Fungi Imperfecti					
***Aspergillus flavipes* (Bain. and Sart.) Thom and Church	X				
***Cladosporium macrocarpum* Preuss				X	
***Fusarium roseum* Lk. emend. Snyd. and Hans. f. sp. *cerealis* (Cke.) Snyd. & Hans. 'Culmorum'					X
4) *Onychiurus cocklei* (Folsom)					
Fungi Imperfecti					
Cladosporium herbarum (Pers.) Link ex S. F. Gray					X
Sporothrix sp.	X				

* Fungi from surface sterilized and nonsurface sterilized Collembola.
** Fungi from surface sterilized Collembola.

(DEWITH and JOOSSE, 1971). It is then assumed that at least 25 % of the treated Collembola also contained empty guts (CHRISTEN, 1974). The molting process probably contributed to a lower number of fungi isolated.

Few fungi grew from surface sterilized specimens of *H. macgillivrayi*. Fungal colonies did not appear from surface sterilized individuals until after 4 days contrasting with unsterilized control specimens which produced colonies as soon as 1 day. This delayed growth may be related to a longer time needed for internally located fungi to germinate or grow to the surface. Most of the control specimens produced fungal colonies which suggests that many of the fungi may have been externally associated; however, certain fungi grew from both surface sterilized and control specimens. As a number of the plated control collembolans remained alive, fungi isolated from their tracks and agar substrate were probably of external origin. *Trichosporon* sp., *Cladosporium herbarum*, *Conidiobolus* sp., and *Fusarium roseum* '*Culmorum*' were isolated from secreted or egested material.

Several potential plant pathogens were isolated. *Fusarium roseum* f. sp. *cerealis* '*Culmorum*' was isolated from Collembola from a wheat field. In

this area the form species *cerealis* causes a foot rot of cereals (COOK, 1968). The isolates of *Fusarium roseum 'Avenaceum'* cultured from *H. macgillivrayi* likewise might be pathogenic to plants. The occurence of ingested and passed plant pathogens could be significant in an area where large populations of Collembola are present. Maintaining, spreading, increasing, or decreasing fungus propagules might result from the activities of Collembola.

Beauveria brongniartii and *Paecilomyces farinosus* appeared pathogenic on *H. macgillivrayi* in the isolation plates. Other fungi, especially *Conidiobolus coronatus, Scopulariopsis asperula, S. brevicaulis, Verticillium lecanii,* and possibly *Chrysosporium pannorum,* are known insect parasites (BATRA *et al.,* 1973; GAMS, 1971; MACLEOD and MÜLLER-KÖGLER, 1973; MORTON and SMITH, 1963), and might also be parasitic on species of Collembola.

The most consistently isolated species of fungi were penicillia, mostly *Penicillium atramentosum* and *P. canescens,* and *Cladosporium herbarum. Penicillium canescens* is of worldwide distribution, but most often recorded from arable soils including semi-arid soils (DOMSCH and GAMS, 1972). *Penicillium astramentosum* is not common, judging from its infrequent appearance in lists of fungi isolated from soil (RAPER and THOM, 1949). In contrasts, *C. herbarum* is ubiquitous and commonly isolated from soil and litter around the world (DOMSCH and GAMS, 1972).

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SUMMARY

Thirty species of fungi were isolated from four species of soil and dung-inhabiting Collembola. Fungi included soil saprophytes, probable collembolan pathogens, and possible plant pathogens. Some of the fungi are probably external associates of Collembola. Others are ingested and digested, or ingested and passed in a viable state.

RÉSUMÉ

Trente espèces de champignons ont été isolés à partir de quatre espèces de Collemboles endogés ou coprophiles. Parmi ceux-ci, il existe des saprophytes endogés, des pathogènes probables envers les Collemboles et peut-être des phytopathogènes. Certains sont probablement associés à la surface du corps des Collemboles. D'autres sont ingérés et digérés ou ingérés et excrétés dans un état viable.

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